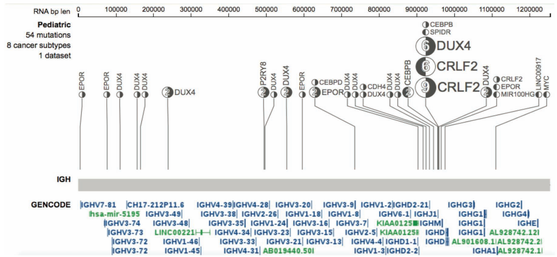
Allelic specificity of immunoglobulin heavy chain (IGH@) translocation in B-cell acute lymphoblastic leukemia (B-ALL) unveiled by long-read sequencing

Liqing Tian[1], Ying Shao[1], Beisi Xu[1], John Easton[1], Xiaotu Ma[1], Yongjin Li[1], Scott Newman[1], Xin Zhou[1], Meredith Ashby[2], Ting Hon[2], Tyson Clark[2], Elizabeth Tseng[2] and Jinghui Zhang[1]
1 Department of Computational Biology, St. Jude Children's Research Hospital, Memphis, Tennessee. 2 Pacific Biosciences, Menlo Park, California.

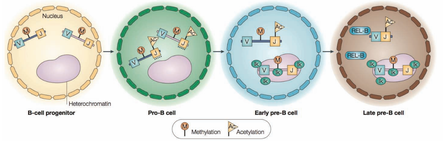
#1485

Background

Proto-oncogenes and IGH translocation is a common mechanism that drives B-ALL.



A stepwise epigenetic process controls immunoglobulin allelic exclusion. The functional Igμ is expressed on the active allele, and the other allele is repressive.

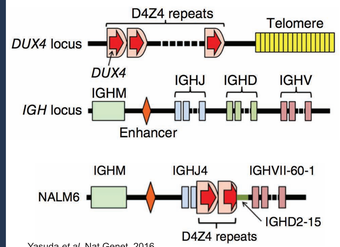


Bergman Y, Cedar H. Nat Rev Immunol. 2004

Rationale

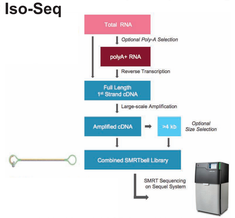
Oncogenic fusion of IGH-DUX4 has recently been reported as a hallmark that defines a B-ALL subtype present in up to 7% of adolescents and young adults B-ALL. The translocation of DUX4 into IGH results in aberrant activation of DUX4 by hijacking the intronic IGH enhancer (Eμ).

How IGH-DUX4 translocation interplays with IGH allelic exclusion was never been explored. We investigated this in Nalm6 B-ALL cell line, using long-read (PacBio Iso-Seq method and 10X Chromium WGS), short-read (Illumina total stranded RNA and WGS), epigenome (H3K27ac ChIP-seq, ATAC-seq) and 3-D genome (Hi-C, H3K27ac HiChIP, Capture-C).



Yasuda *et al*. Nat Genet. 2016

Full-length transcript sequencing by Iso-Seq

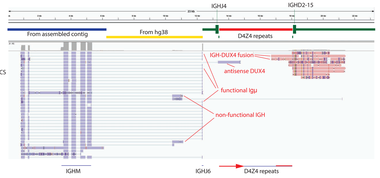


Ashby M *et al*. AACR Annual Meeting. 2017

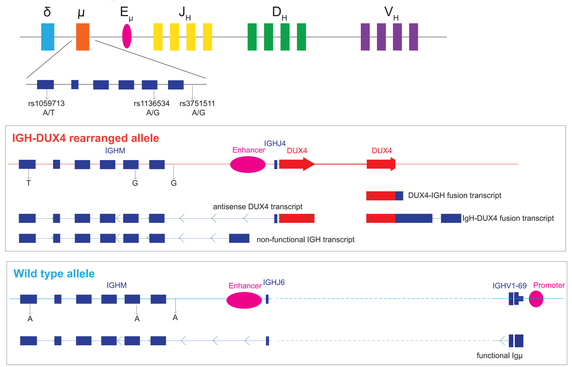
IGH-DUX4: allelic specificity

DNA fragment assembly at IGH-DUX4 translocation region using Chromium WGS and known sequence near breakpoints.

Mapping Iso-Seq full transcript CCS reads (144,756 reads, median length 4,350 bp).



Functional IGH and DUX4 related transcripts are assigned to different alleles accroding to 2 extronic heterozygous SNPs.

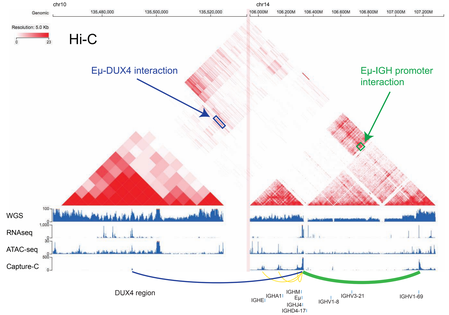


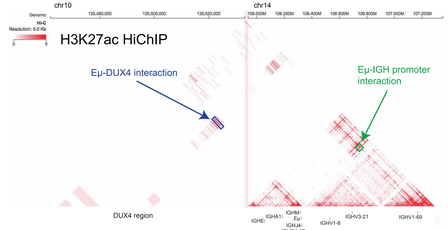
The allele harbored IGH-DUX4 translocation is more repressive than the wild type allele accroding to 3 heterozygous SNPs near IGHM, the constant region of IGH without low mappability issue.

Evidence from 3D genome

Hi-C and H3K27ac HiChIP data confirmed Eμ-IGH promoter interaction and Eμ-DUX4 interaction.

Capture-C was designed to identify possible interactions with Eμ. We estimated the contact intensity of Eμ-DUX4 was ~14-fold lower than that of Eμ-IGH promoter. It may explain the lower expression of DUX4 compared to Igμ.

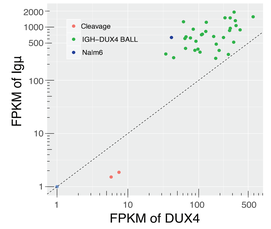




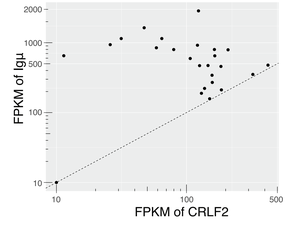
Heterozygous SNPs in the IGH promoter region were uniformly mono-allelic in RNA-seq. In Hi-C, H3K27ac HiChIP, and Capture-C data, Eμ-IGH promoter contact reads were uniformly mono-allelic from the wild type allele.

IGH@ patients

In most B-ALL patients with IGH-DUX4 or IGH-CRLF2 translocation, DUX4/CRLF2 expression is much lower than Igμ.



In 28 out of 32 patients, Igμ to DUX4 FPKM fold>2, median 6.21



In 17 out of 24 patients, Igμ to CRLF2 FPKM fold>2, median 6.54

Conclusion

1 In Nalm6, IGH-DUX4 fusion occurred on the repressive allele that did not express functional Igμ, and the fusion DUX4 exhibited lower expression compared to Igμ.

2 Epigenetic regulation at IGH locus is a plausible explanation as we observed weaker interaction of Eμ-DUX4 than that of Eμ-IGH promoter.

3 It might be the same in B-ALL patients with IGH-DUX4 or IGH-CRLF2 translocation.

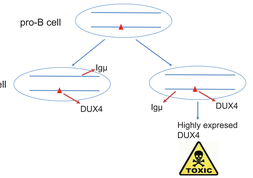
Question: Why does DUX4 translocate to the repressive IGH allele?

Model A:

Highly expressed DUX4 is toxic to pre-B cell and leads to cell death.

"High levels DUX4 induced rapid cell death in 3T3 fibroblasts."

Bosnakovski D *et al*. EMBO J. 2008



Model B:

The allele with DUX4 translocation will loss the ability of successful VDJ recombination and expressing functional Igμ.